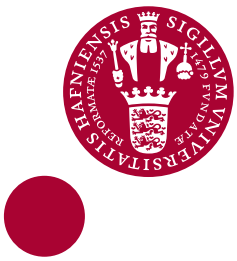

Meal induced gut hormone secretion is altered in aerobically trained compared to sedentary young healthy males

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ORIGINAL ARTICLE

Meal induced gut hormone secretion is altered in aerobically trained compared to sedentary young healthy males

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Abstract Postprandial insulin release is lower in healthy aerobically trained (T) compared to untrained (UT) individuals. This may be mediated by a lower release of the two incretin hormones [glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP)] in T. The aim of this study was to assess and compare gut hormone response and satiety changes after a liquid meal intake in young, healthy T and UT males. Postprandial gut hormone release and subjective feelings of hunger, satiety, fullness and prospective food consumption were assessed before and frequently for the following 3 h after a 200 ml liquid meal (1,260 kJ and 27, 41 and 32 energy % as protein, carbohydrates and fat, respectively) in ten T and ten UT young, healthy male subjects. The insulin and GIP responses were markedly lower in T than UT and correlated during the first 30 min after the liquid meal. Baseline GLP-1 concentration was higher in T versus UT, but the response in the following 3 h after a liquid meal was similar in T and UT. Satiety measures did not differ between groups throughout the test. It is possible that in aerobically T subjects, a lower GIP release is partly responsible for a lower postprandial incretin stimulated insulin secretion.

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Keywords Insulin · Incretin · GLP-1 · GIP · Exercise

Abbreviations

IL-6	Interleukin-6
PP	Pancreatic polypeptide
PFC	Prospective food consumption
T	Aerobically trained
UT	Untrained
VO_2max	Maximal oxygen uptake
FFA	Free fatty acids
DPP-4	Dipeptidyl peptidase-4
GLP-2	Glucagon-like peptide-2
AUC	Area under the curve
BMI	Body mass index
LBM	Lean body mass
GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon-like peptide-1
PYY	Peptide YY
VIP	Vasoactive intestinal polypeptide

Introduction

Endurance training is known to induce adaptations in the cardiovascular system and in skeletal muscle. Thus, maximal oxygen consumption (VO_2max) increases in response to a training program due to an increase in cardiac stroke volume and the skeletal muscle adapts with increased vascularization and mitochondrial content (Holloszy et al. 1964; Holloszy 2008). Likewise, metabolism adapts to the increased substrate turnover by an increased capacity for lipid oxidation and an increased activity of enzymes in the glycolytic and gluconeogenic pathways (Brooks and

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Mercier 1994; Essen-Gustavsson and Henriksson 1984). Sympathoadrenal and hormonal responses to exercise are also influenced by physical training (Galbo 1983; Kjaer and Galbo 1988). With the increasing knowledge of the gastro-intestinal tract as an organ that can produce and secrete a variety of hormones involved in digestion, satiety and energy storage, it could be speculated that the endocrine function of the gut is also influenced by being in a physically trained state.

More than 30 years ago, it was found that gut hormone secretion changes during exercise (Hilsted et al. 1980; Galbo et al. 1975). More remarkably, insulin secretion upon stimulation is diminished in athletes in the resting situation (Dela et al. 1991; Mikines et al. 1989). Thus the β-cell must have some form of "memory" that immediately regulates insulin secretion when a secretagogue is administered and being in a trained state alters this memory. A possible mechanism could be that the intestinal L- and K-cell products, glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), together known as the incretin hormones, are released in lesser amounts, or that the incretin sensitivity in the β-cell is decreased, since both hormones stimulate the glucose-induced insulin secretion (Holst et al. 1987; Dupre et al. 1973).

Previous studies have shown how physical training affects postprandial gut hormone secretion in obese subjects following a 12-week exercise regime (Kelly et al. 2009; Martins et al. 2010, 2013). However, it has not been investigated whether postprandial hormone release is altered in non-obese, healthy subjects that are aerobically trained compared to age and BMI matched sedentary persons.

Acute exercise-induced alterations in appetite-regulating hormone release are compatible with decreased sensations of hunger (e.g., lower acylated ghrelin, higher PYY) (King et al. 2011; Broom et al. 2007, 2009). Also it has recently been shown that an exercise, compared to food-induced energy deficit, creates divergent sensations of hunger and release of appetite regulating hormones up to 9 h after the exercise bout (King et al. 2011). Being in a long lasting aerobically trained state necessarily involves frequent, repetitive bouts of endurance exercise, but whether this leads to more permanent alterations in gut hormone release that could influence appetite regulation is unknown.

The aim of this study was to assess and compare the gut hormone response to a liquid meal in young aerobically trained (T) and untrained (UT) healthy males; secondary to measure how this affected satiety and hunger assessed by visual analog scale (VAS). We hypothesized that gut hormone release in general, and the incretin release in particular, would be lower in the aerobically trained compared to untrained state. Secondarily we hypothesized that T would report lower satiety and higher hunger ratings on the VAS, since the amount of energy ingested would account for a smaller part of their daily energy requirement compared to UT.

Methods

Twenty healthy, young, normal weight males were recruited through notices posted on the Copenhagen University bulletin boards and in student magazines. Participants were divided into an UT group with a sedentary lifestyle, e.g., did not perform any aerobic or resistance exercise, during at least the last 6 months and a $VO_2max < 50$ ml min^{-1} kg^{-1} body weight. The T group was regular aerobic exercise performers doing long distance running, bicycling or triathlon (>three exercise bouts a week) during several years with a $VO_2max > 60$ ml min^{-1} kg^{-1} body weight. Subjects were asked to follow their habitual food intake and lifestyle during the study period. In addition, specific dietary instructions were given to ensure a carbohydrate intake of at least 250 g day^{-1} three days prior to the experiments. Before enrollment subjects gave their informed signed consent. The study was approved by the Copenhagen ethics committee (journal no. H-3-2011-043) and performed according to the declaration of Helsinki.

VO_2max test and body composition

Measurement of VO_2max was performed as a graded exercise test on a stationary ergometer bike (Ergometrics 800, Jaeger, Würzburg, Germany) and oxygen uptake was measured by an online system (Oxycon pro, Jaeger, Würzburg, Germany). Determination of VO_2max followed the leveling off criteria and the respiratory exchange ratio: $\dot{V}CO_2/\dot{V}O_2$ had to be greater than 1.15. VO_2max was calculated as the highest average value obtained during 20 s of exercise. Body composition was measured using a dual-energy X-ray absorptiometry scanner (GE Medical Systems, Lunar iDXA Series, Madison, WI, USA).

Meal test

On the day of the meal test subjects reported to the lab after an overnight fast. Subjects were asked not to perform vigorous exercise for at least 24 h before the test. A venous catheter was inserted in a superficial elbow or hand vein and baseline blood samples were drawn at −10 and 0 min for determination of baseline values. Hereafter a 200 ml

liquid meal (Nutridrink Protein, Nutricia A/S, Denmark) containing 1,260 kJ energy distributed between 20 g protein, 31.2 g carbohydrate and 10.6 g fat was consumed. This provided 27 % of the energy as protein, 41 % as carbohydrate and 32 % as fat. The liquid meal was chosen to ensure a robust intestinal hormone stimulus. Following the liquid meal, blood samples were drawn every half an hour for 3 h.

After the last blood sample was drawn, subjects were offered an ad libitum spaghetti meal (Knorr Spaghetteria Bolognese, Unilever Denmark A/S, Denmark) containing 367 kcal of energy pr. 100 g, distributed between 16 % protein, 75 % carbohydrate, 7 % fat and 2 % dietary fibers. During the ad libitum meal, the plate was continuously filled. The subjects ate until they reported to be comfortably satiated. Water was freely available during the day.

Subjective feelings of hunger, satiety, fullness and prospective food consumption (PFC) were marked on a 100 mm VAS before, every 30 min for 3 h after the liquid meal intake and lastly after the ad libitum meal. The following questions were asked: How hungry do you feel (not hungry at all—as hungry as I have ever felt)? How satiated do you feel (not satiated at all—I can't eat anything)? How full do you feel (not full at all—very full)? How much do you think you can eat (nothing at all—a large amount)?

Biochemistry

Blood samples were collected in chilled EDTA containing tubes for glucose, free fatty acids (FFA), glycerol and Interleukin-6 (IL-6) measurements; in BD P800 (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) vacutainers containing various proteases as well as dipeptidyl peptidase-4 (DPP-4) inhibitor for GIP measurement; in BD EDTA and aprotinin containing vacutainers for galanin, acylated ghrelin, GLP-1 total, GLP-2, glucagon, insulin, motilin, obestatin, pancreatic polypeptide (PP), Peptide YY (PYY), secretin, somatostatin and vasoactive intestinal polypeptide (VIP). Tubes were immediately cooled and centrifuged for 10 min at 2,000*g* and 4 °C. After centrifugation, plasma was stored at −80 °C until time of analysis. All samples were analyzed in duplicates.

Plasma glucose, FFA and glycerol were measured on a Hitachi 912 Chemistry Analyzer (Roche A/S, Hvidovre, Denmark). Insulin (K6219) was measured by a Dako A/S (Elektra-Box Diagnostica ApS, Roedovre, Denmark) ELISA kit. IL-6 (HS600B) was measured by a high sensitivity R&D Systems immunoassay (AH Diagnostics, Aarhus, Denmark). Acylated ghrelin (KT-364), PYY (KT-378), obestatin (KT-495), secretin (KT-27996) and somatostatin (KT-28589) were measured by commercial immunoassays (Kamiya Medical Company, AH Diagnostics, Aarhus, Denmark). Galanin (S-1347) was assessed by a Bachem immunoassay (AH Diagnostics, Aarhus, Denmark). GIP (EZHGIP-54K), PP (EZHPP40K) were assessed by Merck Millipore immunoassays (AH Diagnostics, Aarhus, Denmark). Motilin (BG-HUM11526) was measured by a NovaTeinBio immunoassay (AH Diagnostics, Aarhus, Denmark). VIP (CSB-E08354 h) was measured by a Cusabio immunoassay (AH Diagnostics, Aarhus, Denmark). All above mentioned ELISA samples were read on a Multiskan FC Microplate Photometer (Thermo Fisher Scientific, Slangerup, Denmark). Glucagon, GLP-1 and GLP-2 concentrations in plasma were measured after extraction of plasma with 70 % (glucagon and GLP-1) and 75 % (GLP-2) ethanol (vol/vol, final concentration). The glucagon assay is C-terminal (antibody code no. 4305) and therefore mainly measures glucagon of pancreatic origin (Holst 1982). GLP-1 was measured (Orskov et al. 1994) using antiserum code no. 89390, which is specific for the amidated C-terminus of GLP-1 and therefore mainly reacts with GLP-1 of intestinal origin but also GLP-1 3–36 amide, the primary metabolite (=total GLP-1). For GLP-2 we used antiserum code no. 92160 (Hartmann et al. 2000). The antiserum is N-terminal and therefore measures only active GLP-2 of intestinal origin. For all three assays, sensitivity was below 2 pmol l^{-1}. Intra-assay coefficient of variation was below 6 % at 20 pmol l^{-1}, and recovery of standard, added to plasma before extraction, was about 100 % when corrected for losses inherent in the plasma extraction procedure.

Statistical analysis

Statistical analysis was performed in SigmaPlot 12.0 (Systat Software Inc., Erkrath, Germany). Baseline values on the VAS, of hormone and metabolite concentrations and the amount of food eaten during the ad libitum meal were compared by student's *t* test. Hormone secretion and metabolite changes after the liquid meal intake were compared by two-way repeated measures analysis of variance. Area under the curve (AUC) was calculated using the trapezoidal rule as the total area below the curve. AUC data were compared by student's *t* test. Pearson's linear regression model was employed to assess correlation between insulin and GIP. Data are given as mean ± SE. A *P* value less than 0.05 was considered significant in two-tailed testing.

Results

There was no difference in body mass index (BMI) or fasting glucose between the two groups. As expected T had significantly lower % body fat, higher lean body mass (LBM) and VO_2max compared to UT (Table 1).

Table 1 Subject characteristics

	T	UT
n	10	10
Age (years)	26 ± 1	25 ± 1
Height (cm)	182 ± 2	185 ± 2
Weight (kg)	75 ± 3	75 ± 2
Body mass index (kg m^{-2})	22 ± 1	22 ± 1
Body fat (%)	12 ± 1	21 ± 1*
Lean body mass (kg)	62 ± 2	56 ± 1*
Maximal oxygen uptake (ml min^{-1} kg^{-1})	67 ± 2	42 ± 2*
Fasting glucose (mmol l^{-1})	5.3 ± 0.1	5.5 ± 0.1

Data are mean ± SEM

T trained, *UT* untrained

* ($P < 0.01$) T versus UT by student's *t* test

Substrates, metabolites and insulin

There was no difference in baseline plasma glucose concentration between T and UT, respectively (5.3 ± 0.1 vs. 5.5 ± 0.1 mmol l^{-1}) (Fig. 1). Two-way analysis of variance showed a significant effect of training ($P < 0.05$) and time ($P < 0.05$) on glucose levels. Baseline plasma insulin concentration tended to be lower in T (21 ± 2 pmol l^{-1}) compared to UT (31 ± 5 pmol l^{-1}, $P = 0.053$) (Fig. 1). There was a significant effect of training ($P < 0.05$) and time ($P < 0.05$) on insulin release. Baseline plasma glycerol and FFA concentration (65 ± 8 vs. 102 ± 14 and 407 ± 48 vs. 722 ± 93 µmol l^{-1}) were significantly ($P < 0.05$) lower in T versus UT, respectively. In response to the meal, there was a significant effect of training and time ($P < 0.05$) on both glycerol and FFA concentrations in plasma (Fig. 2).

Incretins

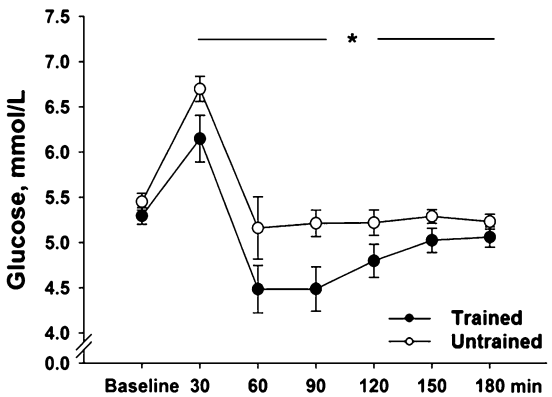
Baseline GIP plasma concentrations were similar in T (47 ± 4 pg ml^{-1}) and UT (56 ± 9 pg ml^{-1}). During the meal, GIP plasma concentrations were lower in T versus UT ($P < 0.05$) (Fig. 3). Baseline GLP-1 plasma concentration was higher ($P < 0.05$) in T (13 ± 1 pmol l^{-1}) versus UT (10 ± 1 pmol l^{-1}). During the meal, plasma concentrations of GLP-1 were significantly ($P < 0.05$) higher in T versus UT (Fig. 3). A significant effect of time ($P < 0.05$) was seen for both hormones. There was no difference in incremental GLP-1 concentrations between T and UT (1,133 ± 185 vs. 828 ± 155 pmol l^{-1}180 min, respectively, $P > 0.05$).

Other L-cell products and IL-6

There was no plasma GLP-2 concentration difference between T and UT at baseline (17 ± 1 vs. 18 ± 2 pmol l^{-1}, respectively) or during the liquid meal (Fig. 3). Likewise, baseline PYY and IL-6 plasma concentrations were similar in T (1.3 ± 0.3 and 0.8 ± 0.1 ng ml^{-1}, respectively) and UT (0.8 ± 0.2 ng ml^{-1} and 0.9 ± 0.1 pg ml^{-1}, respectively) (Fig. 3). There was no difference in PYY and IL-6 between T and UT during the meal test. A significant effect of time ($P < 0.05$) was seen for all three hormones (Fig. 3).

Other gut hormones

Baseline acylated ghrelin was higher and secretin concentrations lower in plasma in T (22 ± 2 fmol ml^{-1} and



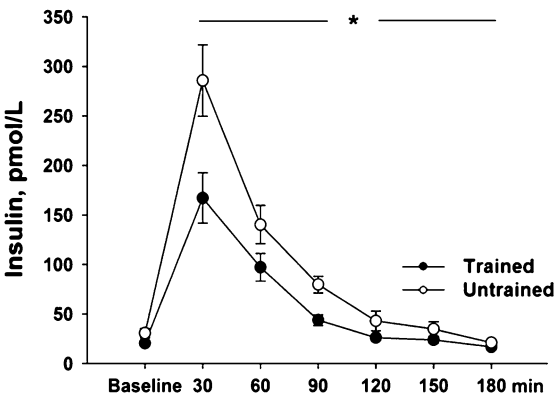


Fig. 1 Glucose and insulin concentrations in plasma 3 h following a liquid meal. T *closed circles* and UT *open circles*. Data are mean ± SEM. Two-way repeated measures ANOVA showed a significant effect of training (*) ($P < 0.05$) and time ($P < 0.01$) on insulin and glucose, with no interactions

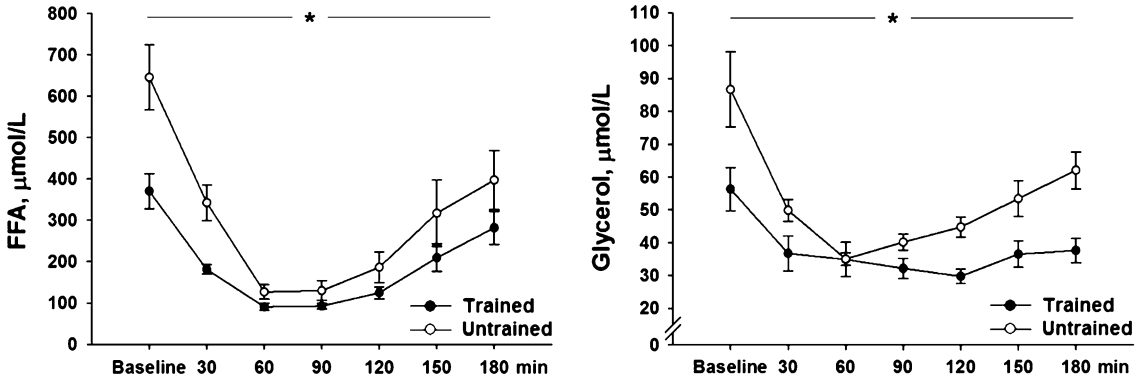


Fig. 2 FFA and glycerol concentrations in plasma 3 h following a liquid meal. T *closed circles* and UT *open circles*. Data are mean ± SEM. Two-way repeated measures ANOVA showed a significant effect of training (*) ($P < 0.05$) and time ($P < 0.001$) on FFA and glycerol, with no interactions

227 ± 12 pg ml^{-1}, respectively) than UT (17 ± 1 fmol ml^{-1} and 305 ± 15 pg ml^{-1}, respectively) ($P < 0.05$), while baseline glucagon plasma concentrations tended ($P = 0.09$) to be lower in T versus UT (8 ± 1 vs. 11 ± 1 pmol l^{-1}, respectively) (Fig. 4). Baseline plasma concentrations of galanin, motilin, secretin, PP, obestatin, somatostatin and VIP did not differ between T and UT. In response to the liquid meal, plasma glucagon and secretin concentrations in plasma were lower in T versus UT ($P < 0.05$), whereas no differences between groups were found for plasma ghrelin, galanin, PP, obestatin, motilin, somatostatin and VIP (Fig. 4). A significant effect of time was seen for plasma ghrelin, glucagon, secretin PP, obestatin, somatostatin and VIP ($P < 0.05$), but not for galanin or motilin.

Appetite score

There was no difference between the groups in hunger, satiety, fullness or PFC on the VAS before, after the liquid meal intake or after the ad libitum meal (Fig. 5). Hunger decreased and satiety increased in the first 60 min and PFC decreased and fullness increased in the first 90 min after meal intake compared to baseline. There was a tendency for a difference in ad libitum meal intake after the meal test between the groups (T: 737 ± 48 vs. UT: 589 ± 59 g, respectively, $P = 0.07$). Excluding a single outlier in the UT group that ate twice the average amount of food compared to his group, there was a significant difference in food intake between the groups (T: 737 ± 48 vs. UT: 541 ± 39 g, respectively, $P < 0.05$).

Discussion

The major finding in the current study is the lower secretion pattern of GIP in aerobically trained compared to untrained, healthy, young males. Thus postprandial GIP release was lower in T than UT, and the secretion pattern of GIP followed the well-known insulin response, which was also reproduced in the present study. The total GLP-1 response was higher in T compared to UT; however, this was clearly explained by the difference in the baseline GLP-1 concentration, so that the incremental response as such was similar. Nevertheless, the baseline values were markedly different and there is no obvious explanation for that. The length of the fasting period was identical for the two groups, being approximately 10 h, and the trained subjects were asked to perform their last exercise bout at least 24 h before experiments.

The present study is cross sectional in design and a prospective intervention study, where ~37-year, obese men and women followed a 12-week exercise program, failed to demonstrate an effect of training on fasting GLP-1 concentration or on meal-induced increase in GLP-1 (Martins et al. 2010). However, in comparison with the present study the subjects were older, obese, and in a catabolic state during the intervention. The fitness level was low and the improvement in VO_2max modest (Martins et al. 2010). In the present study there was a substantial difference in VO_2max and daily training regimen between the groups, but otherwise the groups were similar with respect to age, weight and BMI. With the marked difference in aerobic fitness level between the two groups in the present study, it follows that also the body composition inevitably must be different. This is likely to influence the gut hormone response; however, regardless of which correcting factor is used (LBM or fat mass) either GIP or GLP-1 is lower in T compared to UT (data not shown). Thus differences in body composition alone cannot explain the findings, and when the hypothesis in the current study relates to whether differences between trained and untrained men exists, it would be inappropriate to isolate one characteristic (in casu VO_2max) at the expense of many other (measured and unmeasured) characteristics of the trained state.

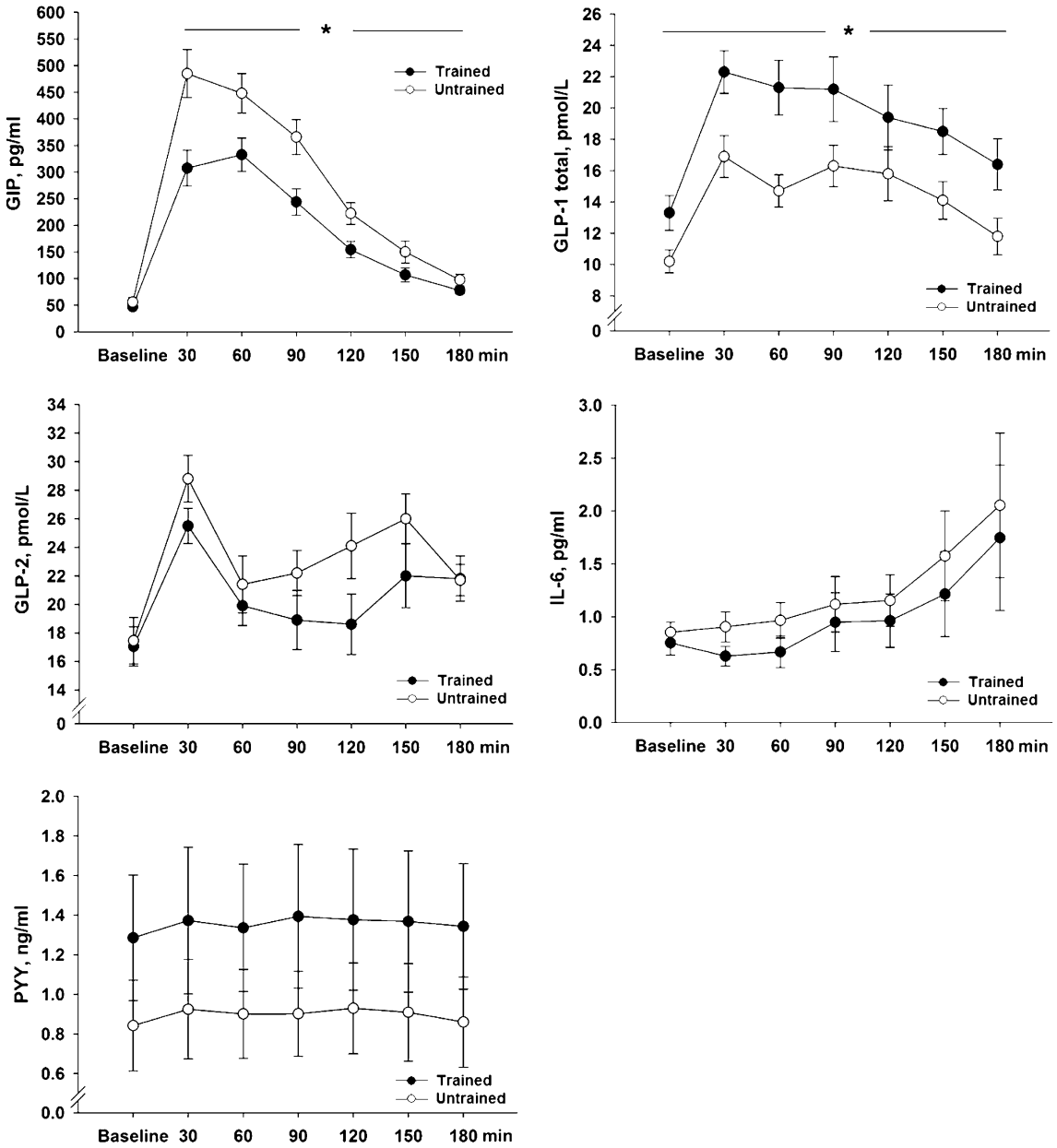


Fig. 3 Glucose-dependent insulinotropic polypeptide (*GIP*), glucagon like peptide-1 (*GLP-1*), glucagon like peptide-2 (*GLP-2*), peptide YY (*PYY*) and interleukin-6 (*IL-6*) concentrations in plasma 3 h following a liquid meal intake. T *closed circles* and UT *open circles*. Data are mean ± SEM. Two-way repeated measures ANOVA showed a significant effect of training (*) ($P < 0.05$) and time ($P < 0.001$) on GIP and GLP-1, but only a significant effect of time ($P < 0.001$) on GLP-2, PYY and IL-6, with no interactions

Plasma concentrations of GIP followed the insulin concentrations in the present study. This is in contrast to two studies in obese, glucose intolerant men (Kelly et al. 2009) and obese women (Martins et al. 2013) in which plasma GIP concentrations in response to an OGTT and a meal, respectively, were similar in the trained and untrained state. Compared with the current study subjects were older, obese, and nutrient composition of the given stimulus was different which might explain the found differences.

Both GLP-1 and GIP enhance the glucose-induced insulin secretion. We observed a diminished insulin response in the face of similar incremental GLP-1 concentrations and lower plasma GIP concentrations in T versus UT after the liquid meal intake. Obviously part of this could be explained by the lower glucose concentration in T versus UT. GIP has been shown to be responsible for a significant part of the glucose-induced insulin release up to glucose levels of ~7 mM (Vilsboll et al. 2003), thus a diminished GIP effect on insulin release may explain part

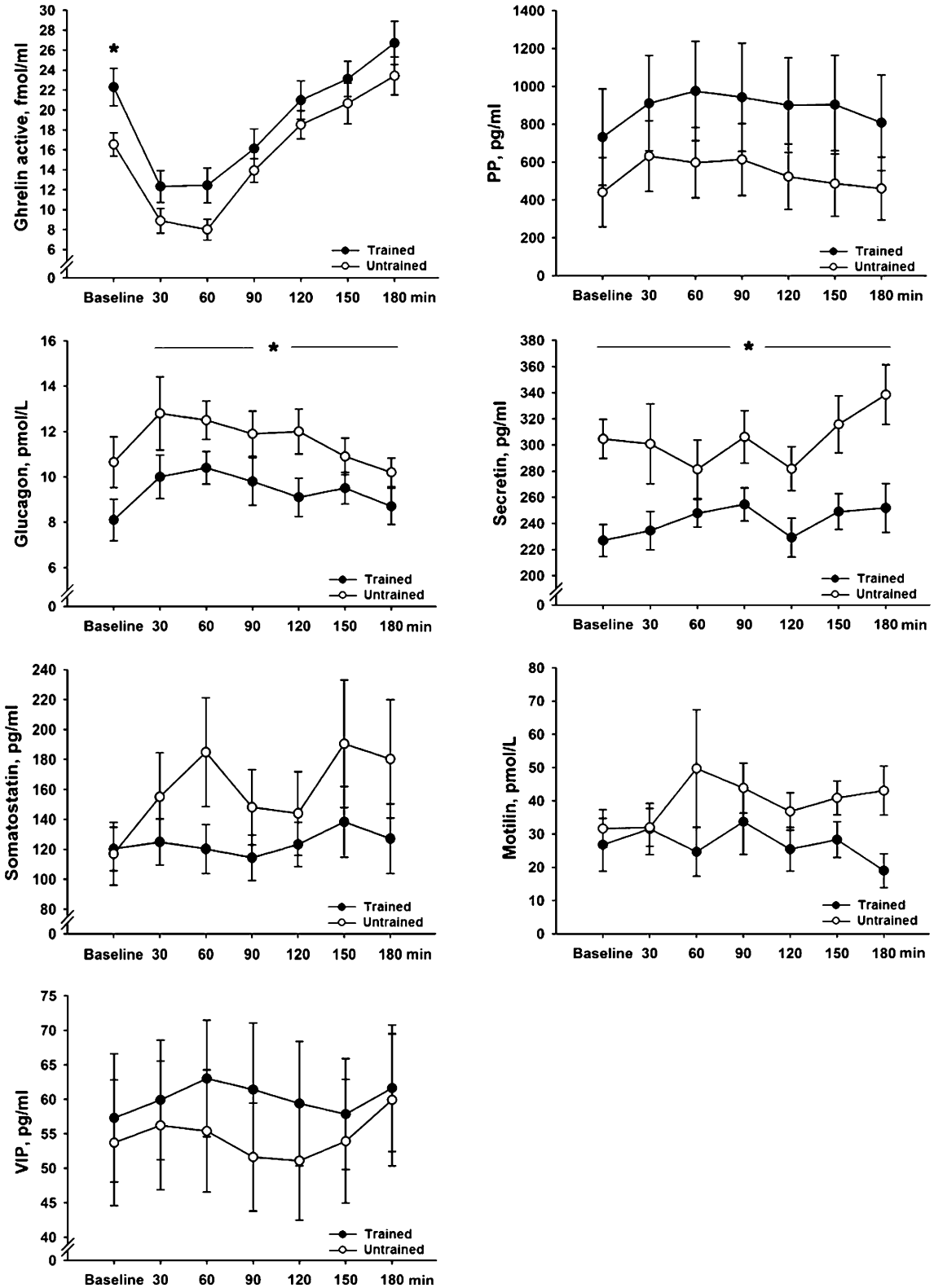


Fig. 4 Galanin, ghrelin, glucagon, motilin, pancreatic polypeptide (*PP*), obestatin, secretin, somatostatin and vasoactive intestinal polypeptide (*VIP*) concentrations in plasma 3 h following a liquid meal intake. T *closed circles* and UT *open circles*. Data are mean ± SEM. Two-way repeated measures ANOVA showed a significant effect of training (*) ($P < 0.05$) and time ($P < 0.05$) on glucagon and secretin, a significant effect of time ($P < 0.05$) on acylated ghrelin, PP, obestatin, somatostatin and VIP, with no interactions. There was no effect of training or time on galanin and motilin and no interactions

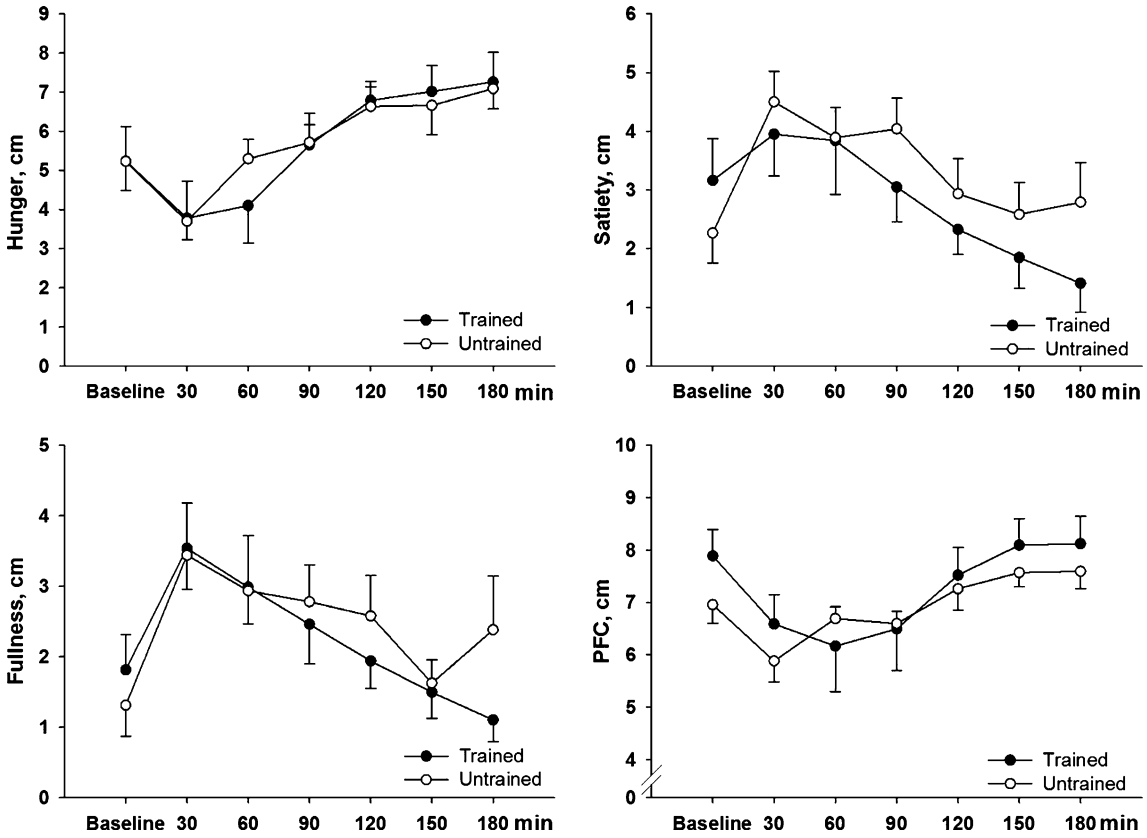


Fig. 5 Hunger, satiety, fullness and prospective food consumption (PFC) measured by VAS 3 h following a liquid meal intake. T *closed circles* and UT *open circles*. Data are mean ± SEM. Two-way repeated measures ANOVA showed no effect of training or time and no interactions

of the lower release in T. This is supported by the fact that plasma concentrations of GIP and insulin correlated positively during the first 30 min after the liquid meal intake (Fig. 6).

In the present study, we did not find a difference in IL-6 between the two groups neither at baseline nor after the liquid meal intake. We did see an increase in plasma IL-6 concentrations after the liquid meal, which might be related to the fat content in the meal (Nappo et al. 2002), but a temporal relationship with GLP-1, GIP or insulin concentrations was absent.

Exercise-induced increase in catecholamine concentrations is a regulatory mechanism for β-cell secretion (Aarnio et al. 2001), and frequent exposure of the β-cells to high sympathoadrenal stimuli results in a down-regulation of insulin secretion upon a given stimulus. This is a likely mechanism by which the β-cells adapt to the trained state. However, whether increased sympathoadrenal activity or parasympathetic activity, via the vagus nerve, also influences GLP-1 release is still being debated (Hansen et al. 2004; Rocca and Brubaker 1999). It should be noted though, that this only has been studied in pigs and rats, not in humans. With a presumably higher vagus activity in T versus UT (e.g., illustrated by a lower resting heart rate), an

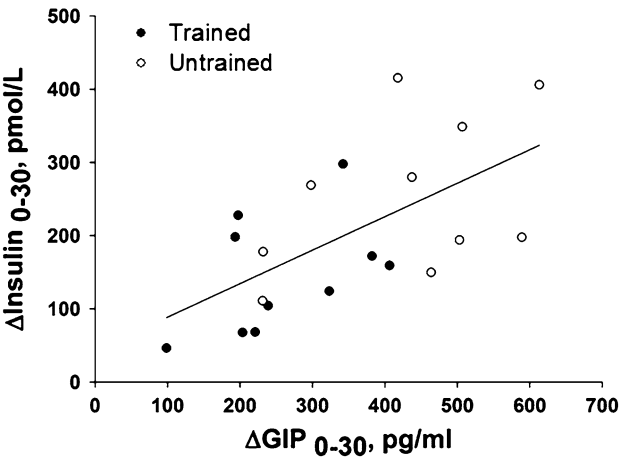


Fig. 6 Correlation between the increases in plasma GIP ($\Delta GIP_{0–30}$) and insulin ($\Delta insulin_{0–30}$) values from baseline to 30 min after a liquid meal intake in ten T (*closed circles*) and ten UT (*open circles*) men. Linear regression showed a significant correlation ($P = 0.004$, $R^2 = 0.38$) between $\Delta GIP_{0–30}$ and $\Delta insulin_{0–30}$

increased vagal tone may be mediating the training effects on the L-cells. Thus the increased baseline plasma concentration of GLP-1 in T versus UT could be due to an increased vagal tone in the former, but a similar increase

would be expected in GLP-2 since the two are co-secreted (Holst 2007). Animal studies seem to indicate that postprandial plasma GIP release is unaffected by vagus tone, though a higher baseline value is seen after vagotomy (Greenberg and Pokol-Daniel 1994).

With the increased energy expenditure during exercise and expected higher resting metabolic rate due to increased LBM (Caudwell et al. 2013; Ravussin et al. 1986), the daily energy requirement and thus food induced stimulation of gastro-intestinal hormonal secretion must be higher in T versus UT subjects. How this affects the L- and K-cells are not known. However, an earlier study investigating the difference in insulin secretion in highly trained and sedentary subjects, has showed that when the amount of glucose ingested is adjusted for the subjects individual daily carbohydrate intake, insulin release is the same in the two groups (Dela et al. 1991). Thus it may be that the β-cell in T individuals loses some of its glucose sensitivity. Whether part of this loss actually could be attributed to lower incretin release or lower incretin sensitivity in the β-cell is unknown.

Both GLP-2 and PYY, like GLP-1, are L-cell products. However, in spite of this we observed a mixed response after the liquid meal intake in T versus UT subjects. Thus, baseline GLP-1 was higher in T versus UT, while GLP-2 and PYY were not different between T and UT subjects. A response to the meal was seen in all three hormones, but only baseline GLP-1 was significantly different between groups and since GLP-1 and -2 are co-secreted this seems paradoxical (Holst 2007).

Ghrelin increases with weight loss (Romon et al. 2006; Scheid et al. 2011; Leidy et al. 2004), and temporarily decreases during and after a single bout of exercise (Broom et al. 2007). In the present study, a higher baseline acylated ghrelin concentration in T versus UT was found. This is most likely explained by the lower fat mass in T compared to UT (Tschop et al. 2001). No postprandial difference between the two groups existed though, and it thus seems that ghrelin release is not affected by the aerobically trained state. Glucagon release is inhibited by GLP-1 (Orskov et al. 1986). Thus a lower glucagon concentration in T compared to UT seems plausible. Also glucagon release has been shown to be increased in subjects with low peripheral insulin sensitivity (Faerch et al. 2009), and since increased peripheral insulin sensitivity is one of the cardinal changes with endurance exercise (Dela et al. 1992) our results support this notion. Secretin is released when duodenal pH decreases. Both baseline and postprandial secretin concentrations were lower in T compared to UT. Strenuous exercise has been shown to increase secretin levels (O'Connor et al. 1995), this stands in contrast to our results and further studies are needed to elucidate this relationship. Galanin, motilin, obestatin, PP, somatostatin and VIP did not differ between T and UT. We have previously found similar plasma concentrations of PP and somatostatin in T and UT men (Dela, unpublished data). Obestatin and motilin releases do not change during acute exercise (Soffer et al. 1993; Manshouri et al. 2008) and we did not find postprandial difference in either of the two hormones. Galanin and VIP concentrations on the other hand may increase during and shortly after exercise (Legakis et al. 2000; Hilsted et al. 1980; O'Connor et al. 1995), however, our data does not support that being in a trained state should alter their release.

Subjective feelings of hunger, satiety, fullness and PFC were assessed before, after the liquid meal intake and after the ad libitum meal. We found no difference in any of the asked parameters between the two groups. Despite this T subjects ate on average 20 % more than UT during the ad libitum meal. This is most likely explained by the greater requirement for energy intake in the trained state. Thus the VAS results do not correlate with the level of fitness or energy intake during the ad libitum meal. This is in line with some (King et al. 1997; Martins et al. 2007) but not all (King et al. 2008) previous studies.

Limitations to the study

Concentrations of obestatin, PP and secretin were higher and PYY lower compared to what has been published before. This is probably a function of the specificity of the antibodies in the commercial assays employed. However it is possible, that a more robust response in PYY would be seen after a solid meal with higher energy content, than used in the present study. The cross-sectional nature of the present study dictates that interpretations of the results should be conservative and the differences in VO_2max, fat and lean mass between the two groups make us unable to pinpoint a single factor explaining our findings. Thus a randomized control study is needed to confirm our findings.

Conclusion

We have demonstrated that the secretion pattern of hormones from the stomach, pancreas, duodenum, proximal- and distal small intestine are different in T compared to UT individuals in response to a mixed liquid meal. Most interestingly postprandial GIP release was lower and incremental GLP-1 release unaltered in T compared to UT. A lower GIP release may therefore be partly responsible for the diminished insulin secretion seen in T.

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Conflict of interest The authors declare that there is no conflict of interest associated with this manuscript.

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